

(12) UK Patent Application (19) GB (11) 2 353 632 (13) A

(43) Date of A Publication 28.02.2001

(21) Application No 0016568.8

(22) Date of Filing 05.07.2000

(30) Priority Data

(31) 19930894

(32) 05.07.1999

(33) DE

(71) Applicant(s)

Bruker Daltonik GmbH
(Incorporated in the Federal Republic of Germany)
Fahrenheitstraße 4, D-28359 Bremen,
Federal Republic of Germany

(72) Inventor(s)

Gokhan Baykut
Jochen Franzen

(74) Agent and/or Address for Service

W H Beck, Greener & Co
7 Stone Buildings, Lincoln's Inn, LONDON, WC2A 3SZ,
United Kingdom

(51) INT CL⁷

H01J 49/42

(52) UK CL (Edition S)

H1D DMG D21B D21X

(56) Documents Cited

GB 2301704 A

GB 2263578 A

WO 98/06481 A

US 6011259 A

US 5572022 A

(58) Field of Search

UK CL (Edition R) H1D DMD DMG

INT CL⁷ H01J 49/42

ONLINE: EPODOC, WPI, JAPIO

(54) Abstract Title

Method and device for controlling the filling of ions into an icr mass spectrometer

(57) The invention relates to a method and a device for controlling the number of ions in an ion cyclotron resonance (ICR) mass spectrometer, whereby the ions enter a multipole ion guide 8 after their formation at a sample 1 e.g. by MALDI or electrospray methods of production, and are stored there temporarily. By measuring the ion numbers in a predetermined subset, the total number of ions in the ion guide may be determined. This information allows the number of ions transferred into the ICR trap 18 for mass spectrometric analysis to be regulated. A mode of the multipole ion guide can ensure that undesirable mass ranges are filtered out before the transfer of ions into the ICR mass spectrometer. The invention makes it possible to eliminate space charge effects, which are caused by over filling the ICR traps.

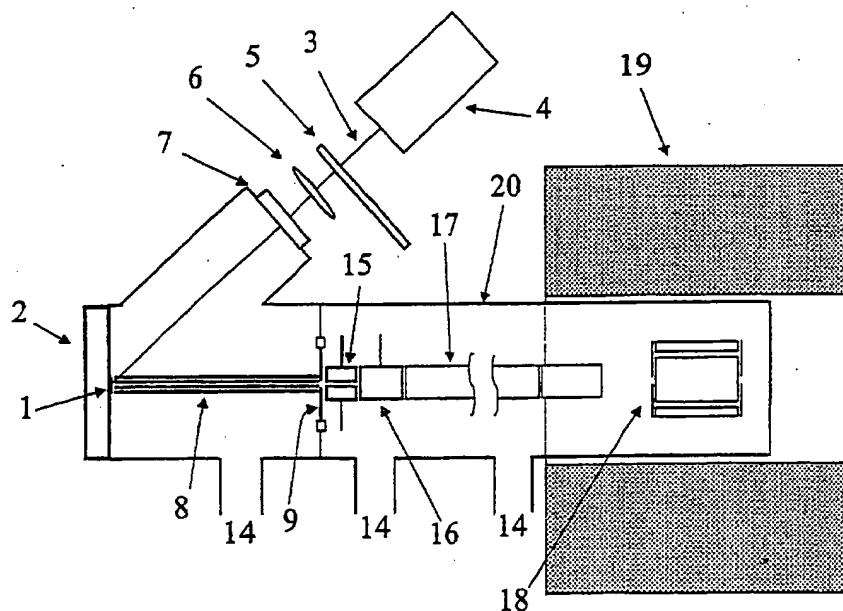


Figure 2

BEST AVAILABLE COPY

GB 2 353 632 A

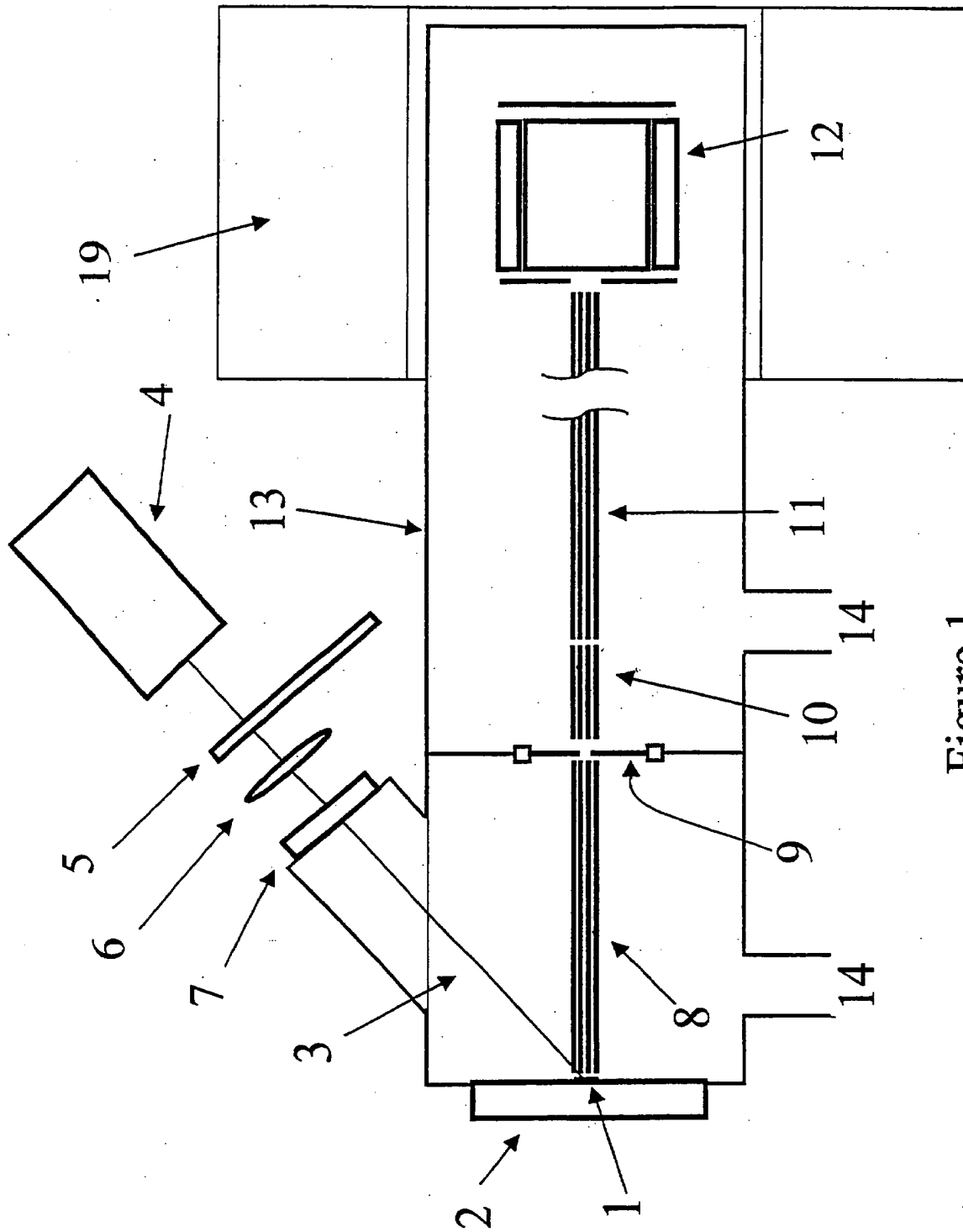


Figure 1

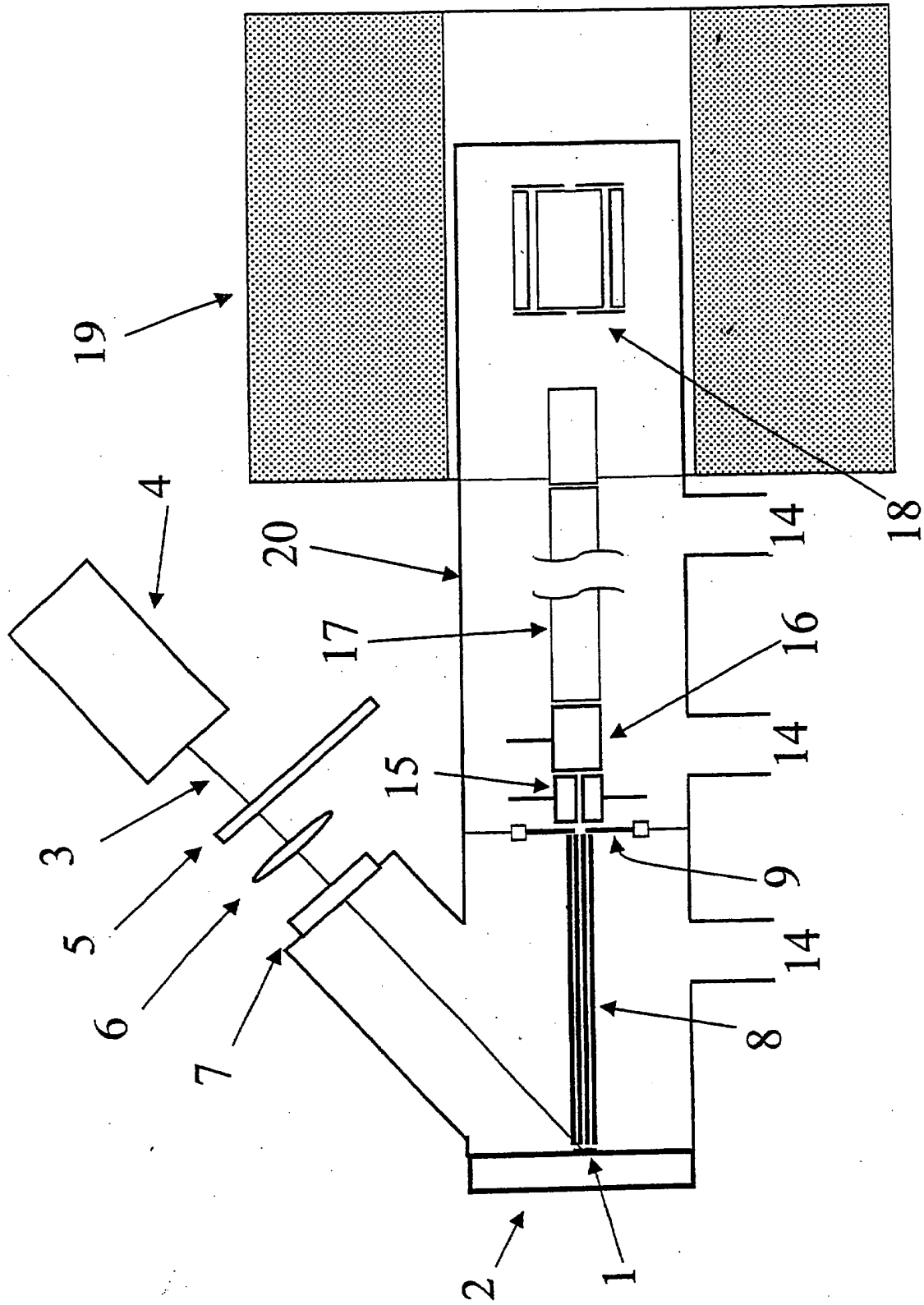


Figure 2

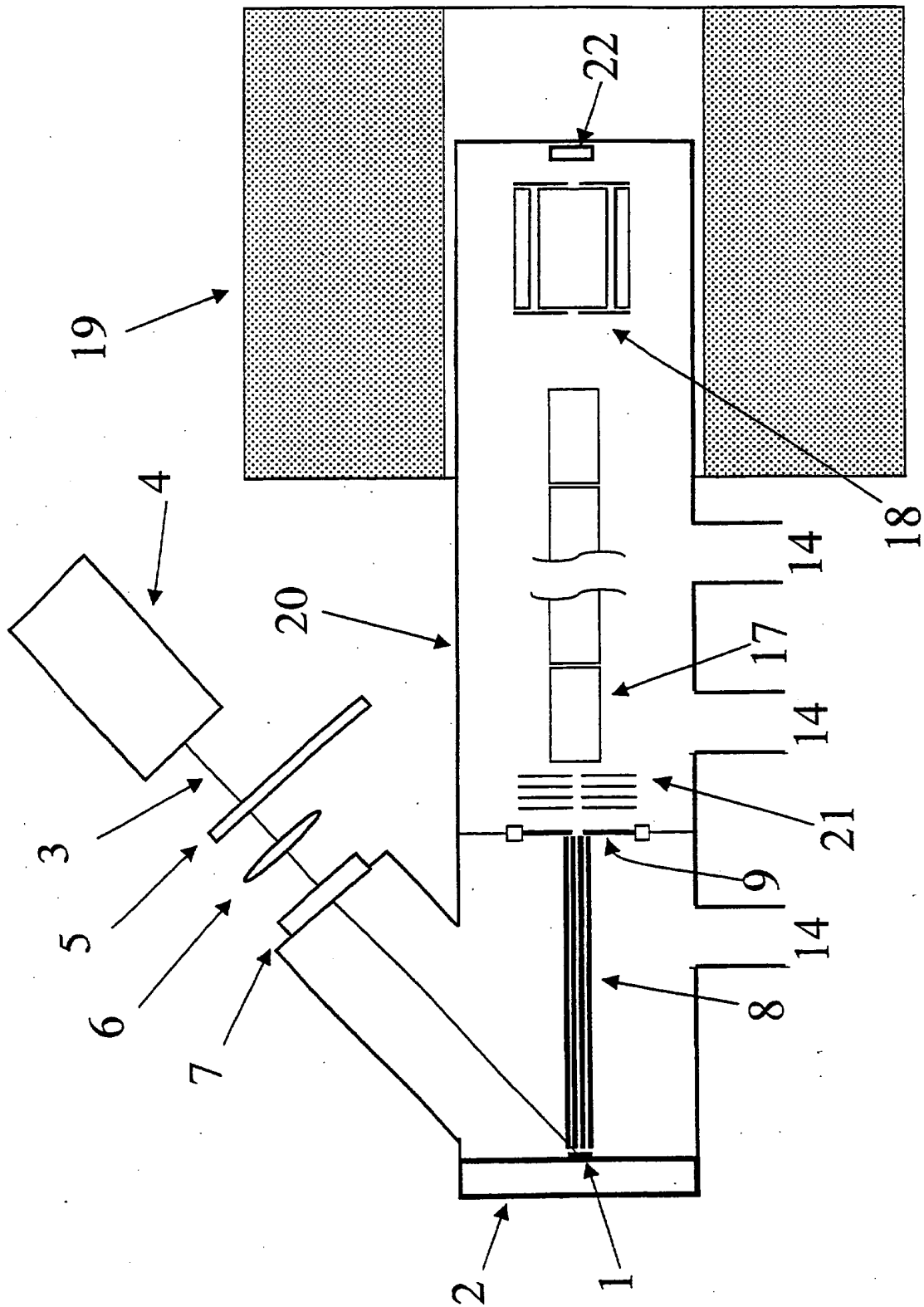


Figure 3

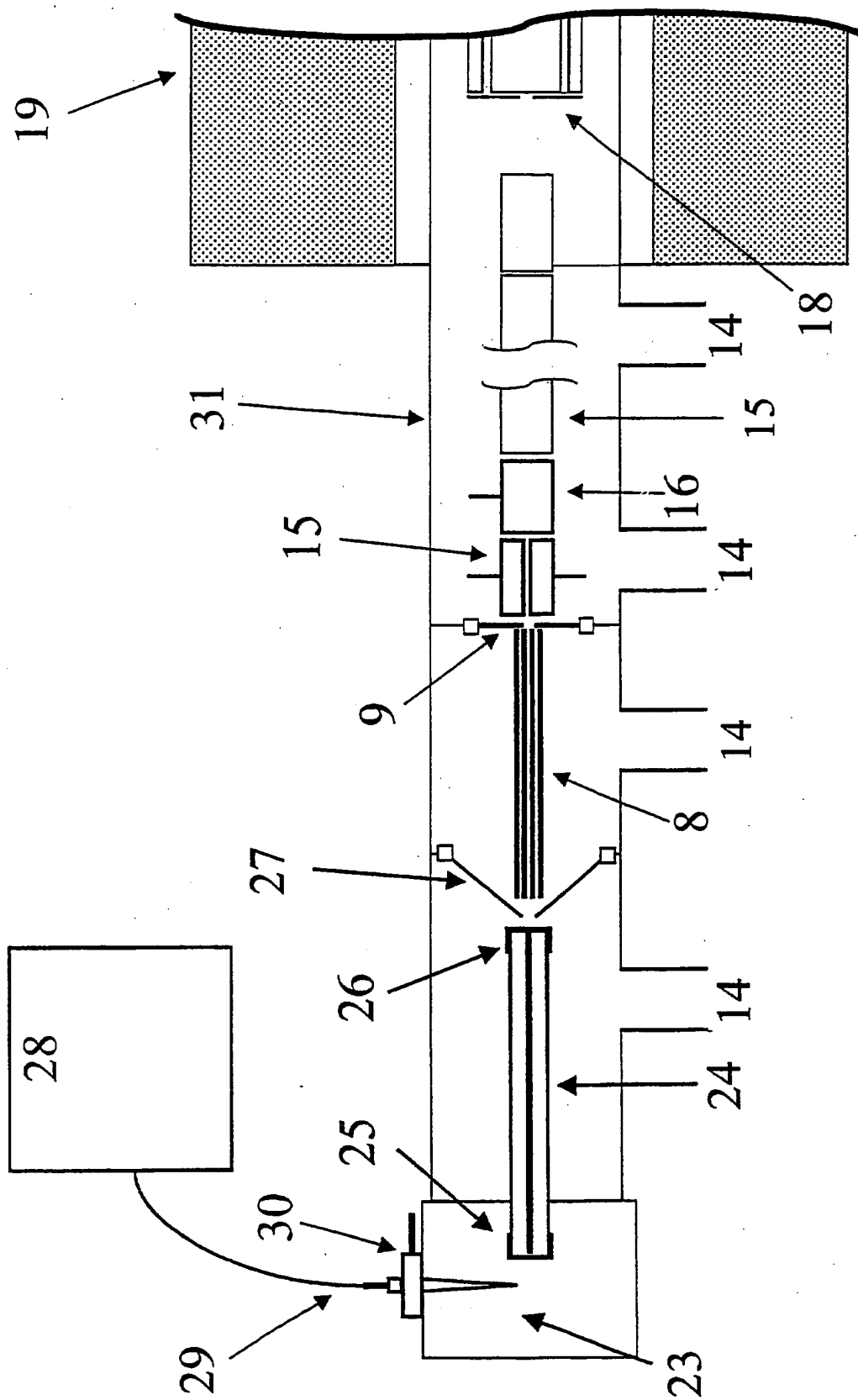


Figure 4

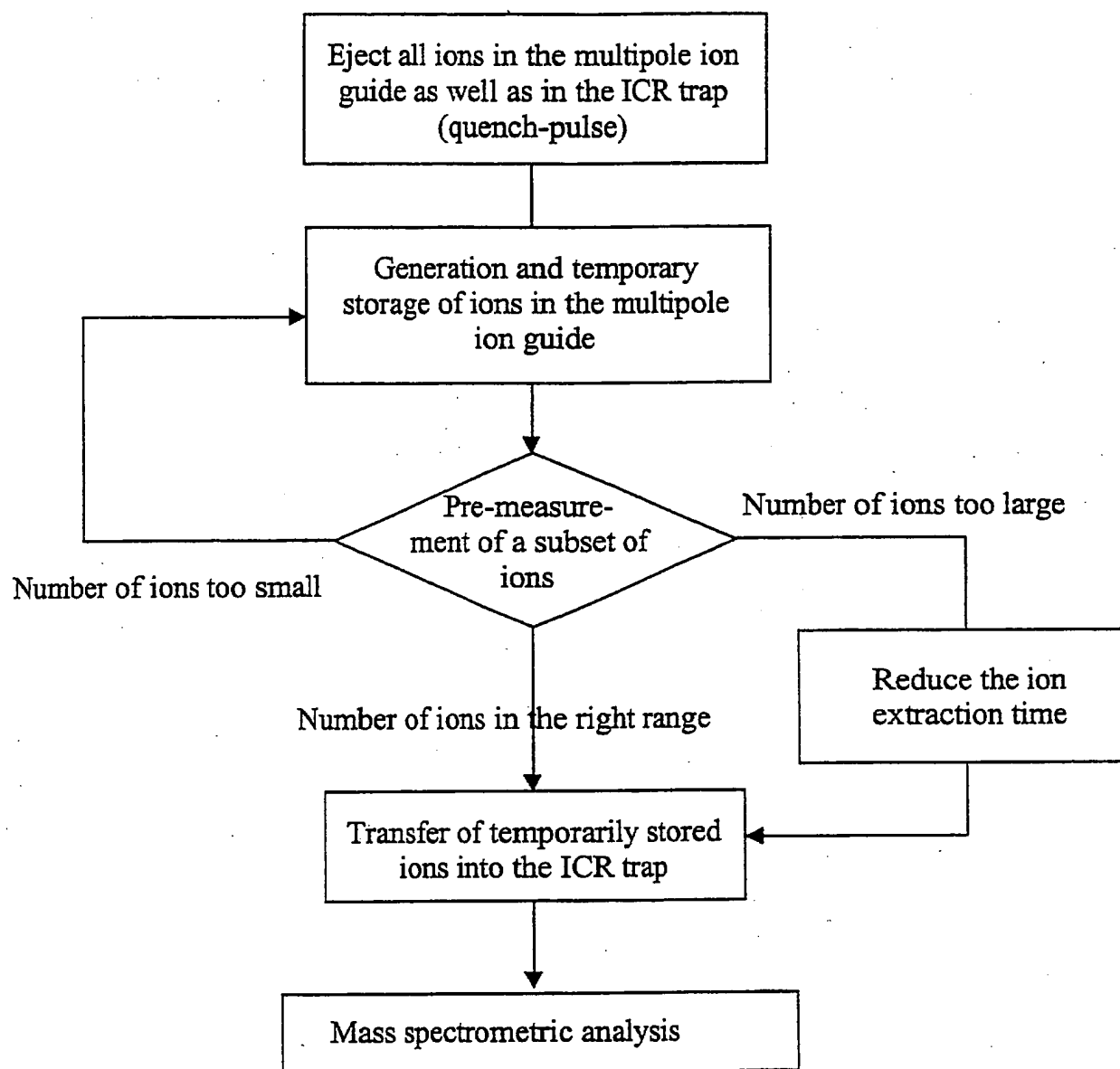


Figure 5

Method and Device for Controlling the Number of
Ions in Ion Cyclotron Resonance Mass Spectrometers

The invention relates to a method and a device for
5 controlling the number of ions in ion cyclotron resonance
(ICR) mass spectrometers.

Conventional methods of ionising substances for mass
spectrometric analysis, such as electron impact, cannot be
applied to large organic or biomolecules. These species can
10 neither be transferred into the gas phase by thermal energy
supply without being decomposed, nor can they be ionised by
electron impact without being fragmented. Contemporary mass
spectrometry very frequently uses electrospray or matrix
assisted laser desorption ionisation (MALDI), which offer
15 much milder ionisation conditions for large molecules.

Electrospray ionisation is probably the most frequently
used ionisation method for the large molecules. A review
article about the mechanism of the electrospray ionisation
was published by P. Kebarle and L. Tang in „Analytical
20 Chemistry“ 65, 972A-986A (1993). Using this method ions are
generated at atmospheric pressure under the influence of
high voltage (3-6kV) between an electrospray needle and a
counter electrode. Although the spray process is often
supported by a slow and fine adjustable syringe pump, the
25 separation of the small charged droplets as a result of the
high ion density on the liquid surface (Coulomb repulsion)
is the primary driving force of the spray process. A drying
gas that flows in counter current to the flight of the
charged droplets leads to the evaporation of the solvent
30 (desolvation process) and thus to the reduction of the
droplet radii. Due to the increasing Coulomb repulsion

forces the ionised molecules are evaporated and often multiply protonated. These ions are transferred through a capillary through a multi-stage vacuum system and through a multipole ion guide into the mass spectrometer for measurement. Electrospray ionisation at atmospheric pressure has dramatically simplified linking separation methods, such as liquid chromatography or capillary electrophoresis, directly to the mass spectrometry.

Laser desorption ionisation (LDI) is has long been used successfully to transfer large organic molecules into the gas phase and to ionise them. A special kind of LDI is the matrix assisted laser desorption ionisation (MALDI). The review article by E. J. Zaluszczyk, D. A. Gage, J. T. Watson in Protein Expression and Purification 6, 109-123 (1995) reports MALDI applications for characterisation of proteins and peptides. A MALDI paper by H. J. Räder and W. Schreppe about the analysis of synthetic polymers with the aid of time of flight mass spectrometry can be found in Acta Polymer. 49, 272-293 (1998).

In MALDI the analyte molecules are mixed with a so-called matrix. The molar ratio of the matrix to the analyte is usually $1:10^2$ to $1:10^4$. The energy of the laser beam is absorbed by the matrix molecules and transferred to the analyte molecules. The latter thus obtains the necessary energy to undergo transition to the gas phase and thereby become partially ionised. The ionisation mostly happens in form of a proton acceptance. Compounds that are used as matrix are mostly proton donors. In special cases, alkaline metal salts or silver salts can be used as additives in order to achieve a corresponding metal ion attachment.

In classical cases of MALDI time of flight mass spectrometry, ions are extracted out of the source region

using a high voltage pulse and accelerated into the flight tube. Contrary to the MALDI time of flight mass spectrometry, in high RF ion traps (Paul traps) and electromagnetic ion traps (Penning traps, ion cyclotron resonance and Fourier transform ion cyclotron resonance mass spectrometry) it is desirable to generate low-energy ions, in order to be able to capture them in the corresponding ion trap without sustaining any losses. Consequently, ions are not accelerated to energies of several kilo electron volts.

In the low energy extraction of MALDI generated ions, the variation of excess energy between the ions becomes more evident and causes difficulties even during capturing these ions. It leads to a considerable fluctuation of the generated mass signals and therefore to irreproducible analytical results. A low voltage MALDI ion source is described by A. N. Krutchinsky, A. V. Loboda, V. L. Spicer, R. Dworschak, W. Ens, K. G. Standing in "Rapid Communications in Mass Spectrometry" 12, 508 - 518 (1998), where the ions are desorbed directly into a quadrupole. Since the ions are practically formed in the quadrupole they are efficiently captured. However, in this method the ions are not collected and trapped in the quadrupole. The quadrupole is used solely as an ion guide in order to transfer ions into the time of flight mass spectrometer.

One of the important differences between the ion trap mass spectrometry and ion transmission mass spectrometry is caused by the limited ion storage capacity of ion traps. Overloading an ion trap is as undesirable as having an insufficient number of ions. Methods for controlling the number of ions in RF traps are described in US-A-5,107,109, DE-A-43 26 549. These patents describe the controlled

generation of ions by electron impact in the trap by regulating the ionisation in the ionisation time of the analyte molecules. In the first case the number of ions is determined by a pre-measurement of the ion charge in the trap and regulated in the immediately following measurement. In the second patent, the actual value of the number of ions extrapolated from integration of several of the preceding mass spectrometric measurements and used for the control. US-A-5,739,530 also describes a controlled ion filling from a multipole ion guide system to quadrupole ion traps.

There are fundamental differences between the pulsed generation of ions by MALDI or LDI and ion formation in a continuous-operating ion source. In the first case, the ionisation is triggered by individual laser pulses, which transfer the small molecules in a crystalline (or liquid) matrix into the gas phase and ionise them in part. During every laser pulse the surface of the sample is also modified and rearranged, and small cradles are formed, while part of the matter is eroded from the surface. As a result the "ion picture" of the next laser pulse is not necessarily the same as the one from the preceding pulse. That is, the number of ions transferred into the gas phase, as well as the intensity ratio of analyte ions to the matrix ions can vary significantly from laser pulse to laser pulse. Consequently, a varying space charge is caused in the trap.

The determination of the ion mass in the FTICR trap is performed by a frequency measurement. Due to the space charge in the trap, this frequency shifts. Therefore, a "reduced cyclotron frequency" is measured, which depends on the strength of the space charge. The publication of J.

B. Jeffries, S. E. Barlow and G. H. Dunn, International Journal of Mass Spectrometry and Ion Processes 54, 169-187, (1983) describes these space charge mass shift effects theoretically. If the number of ions varies from scan to scan and are not regulated, this can cause a corresponding shift in the mass signal each time.

At high ion densities, another undesirable phenomenon appears, the so called "peak coalescence". Signals of ions with a very small mass difference, approach each other and finally coalesce. The product of this coalescence is usually another sharp peak. In MALDI-FTICR mass spectrometry peak coalescence phenomena are frequently observed due to uncontrolled number of ions which are transferred to the ICR trap.

If several scans have to be added up in order to increase the signal-to-noise ratio, these frequency shifts lead to problems. The varying number of ions of two consecutive ionisation processes (e.g. MALDI) produces a varying space charge and varying mass shifts in each acquired spectrum. When adding up spectra, this effect presents itself as a peak broadening and thus leads to a loss of the mass resolution. Nowadays, the FTICR mass spectrometry is used largely because of its extremely high mass accuracy and mass resolution. Therefore, even very small mass shifts caused by space charge effects mean a substantial loss in performance.

Although the difficulties with the variation of the mass spectrometric signal show up particularly in the ionisation method MALDI, it is also observed during other ionisation methods. The electrospray ionisation also shows considerable fluctuations of ion formation. In addition, the electrospray technique allows coupling with

chromatographic or electrophoretic separation methods, which in turn cause very strong time dependent concentration changes (in e.g. chromatographic peaks) and have to be balanced out.

- 5 For this reason it is evident that a control of the space charge in ICR traps is extremely important.

The invention seeks to provide a method and a device for avoiding substantial ion number variations, caused by fluctuating generation of ions and the associated space
10 charge effects in trap mass spectrometers.

The invention provides a method for controlling the filling of the ion trap of an ion cyclotron resonance mass spectrometer used for mass spectroscopic analysis, which method comprises storing the ions temporarily in a
15 multipole ion guide, determining the filling level of the ion guide by measuring a subset of the temporarily stored ions, and thereafter controlling the filling of the ion cyclotron resonance trap from the ion guide for the mass spectrometric analysis in dependence on the filling level
20 of the ion guide.

In order to store the ions in the multipole ion guide system temporarily, it is necessary to apply reflection potentials to the beginning and to the end of the multipole ion guide system. This can be performed using apertured
25 plates. In this context, so-called "skimmers" may be considered as apertured plates. At the entrance end, the MALDI sample carrier plate can provide the potential for trapping the ions. The potential at the exit end must be switchable in order to be able to switch from storage mode
30 to extraction mode.

The filling of the ion trap from the ion guide depends on the number of ions in the ion guide, the so-called level of filling. In general, the filling rate depends on the level of filling. The multipole ion guide used for storage can
5 consist of several individual multipole systems.

When ionisation is by MALDI, the temporary storage of the ions in the multipole ion guide has the additional advantage that ions produced by several individual laser shots can be accumulated in the multipole guide and then
10 transferred into the ion trap. In this way if the analyte concentration is too small, the analyte ions can be enriched by using several laser shots. On the other hand, if there are too many ions produced, this can be established by the preliminary measurement, and considered
15 during the ion trap filling process, in order to keep the space charge under control.

Another advantage of this method is that ions in the multipole ion guide lose their excess kinetic energy within a short time period by collisions with gas molecules if the
20 multipole ion guide is in a section of the mass spectrometer with a slightly elevated pressure. As a result, the probability of capturing the ions in the ion trap mass spectrometer improves.

Furthermore, with the aid of the multipole ion guide,
25 undesirable ions can be filtered out before they are transferred to the ion trap for mass analysis. The presence of undesirable ions just generates additional space charge. Filtering ions in a multipole with rod-shaped electrodes is a well known process. Each multipole has a low mass cut-off
30 limit which depends only on the electrical and mechanical parameters of the multipole.

A number of preferred embodiments of the invention will be described in detail with reference to the accompanying drawings in which:-

Fig. 1 illustrates a laser desorption ion source, where a
5 multipole ion guide is placed directly in front of the sample carrier. Ions which are desorbed from the sample by laser irradiation directly enter the multipole ion guide and are temporarily stored there. A subset of these ions is extracted from the first multipole, which works as a trap,
10 and transferred to the second multipole ion guide, which is temporarily operated as ion detector. This way, the number of temporarily stored ions is determined, and thus the decision is made how many of the remaining ions in the temporary storage will be transferred to the ICR trap for
15 mass spectrometric analysis, or if new laser pulses are necessary to generate more ions.

Fig. 2 shows an LDI source for an ICR mass spectrometer where a storing multipole ion guide is placed in front of the sample carrier. This ion source is equipped with a
20 deflector and an ion detector for determining the number of the ions stored temporarily in the multipole ion guide.

Fig. 3 shows an LDI source, where a storing multipole ion guide is placed in front of the sample carrier. Ions generated by laser irradiation directly enter the multipole
25 and are stored there temporarily. Subsequently, a subset of these ions is extracted by applying a short pulsed potential to the apertured extraction electrode. In this arrangement, the ions fly all the way through the ICR trap and finally hit an ion detector for determining the number
30 of ions stored temporarily (in the multipole) for the succeeding controlled filling the ICR trap.

Fig. 4 shows a device for an FTICR mass spectrometer with and electrospray source and a liquid chromatograph. The components of the substance mixture which are separated with the by the chromatograph, are ionised in the
5 electrospray ion source and transferred to a multipole ion guide with temporary storage capability. The pre-measurement of a defined subset of the temporarily stored ions allows the ion number regulation in the ICR trap.

Fig. 5 is a schematic representation of an algorithm of a
10 procedure for the regulation of the number of the ions, that operates with temporary storage of the generated ions.

Fig. 1 shows a MALDI source combined with an FTICR mass spectrometer. Ions are generated from the sample (1) on the sample carrier (2) by the beam (3) of the laser (4). In
15 this arrangement the laser beam (3) passes through in an adjustable attenuator (5) a focusing lens (6) through the laser window (7) hits the sample (1) in the vacuum system of the mass spectrometer. Ions generated in this way by laser desorption or by MALDI are received directly by the
20 multipole ion guide (8).

The multipole ion guide in this example is an octopole and placed between the sample carrier plate (2) and an apertured plate (lens) (9), so that the multipole ion guide can be used as a multipole ion trap for temporary storage
25 of ions. This apertured plate (9) is electrically insulated against the wall of the pumping stage. The electrical insulation is shown in Figure 1 as small square shaped dots. If positive ions are desorbed during the LDI process, both the sample carrier plate (2) and the end plate (9) are
30 at positive potentials (usually 5-20Volt). Thus, the desorbed ions are kept in this multipole ion trap and stored there temporarily.

Ions that are desorbed with multiple successive laser shots can be accumulated in this linear multipole ion trap. After a certain storage time the stored ions are extracted out by applying a negative potential (usually 1-5 V) to the end
5 plate (9) for a short period of time, after which they follow a path through two other multipole ion guides (10) and (11) (which are in this example octopoles) to the ion cyclotron resonance trap (12) placed in the vacuum system in the magnet (19). The vacuum system (13) consists of
10 differentially pumped stages with separate pumping connections (14).

For regulating the number of ions, this arrangement is used as follows. A small subset of ions (approximately 5-10%) of the ions temporarily stored in the multipole ion trap (8)
15 is transferred using a short pulse to the second short multipole ion guide (10), which is temporarily operating as an ion detector, and "pre-measured" there. Using this measurement, the number of the remaining ions in the multipole ion guide can be calculated. If this number is
20 too large for a normal operation of the ICR trap, and if it can cause difficulties due to the space charge effects, only a certain amount of these ions will be transferred with the extraction pulse into the ICR trap and analysed there. For the regular ion transfer process from source
25 into the ICR trap, the multipole (10) is back to its normal operation mode as an ion guide. If the calculated number of the remaining temporarily stored ions is too small in order to obtain decent signal intensities, in this case further laser pulses are initiated and the whole procedure repeated
30 until the ion number reaches a desired magnitude. Then the accumulated ions are transferred into the ICR trap.

The optimum ion number for the ICR trap must be known in order to apply this method.

A method based on the present invention can also be performed with the aid of a pre-detector. A part of the ions, which were temporarily stored in the multipole ion guide, is extracted using a short electrical pulse (a weak voltage is applied to the extraction plate of the multipole ion guide) and transferred to a predetector. The optimum length of this electric pulse is determined experimentally in such a way, that no more than 5-10% of the total number of ions in the temporary storage extracted. The purpose of the predetector is to convert this short ion pulse into an electrical current pulse, which indicates the "filling level" of the temporary storage. The filling time required for the transfer of the desired number of ions into the ion trap mass spectrometer is determined by a calibrating the signal indicating the filling level of the temporary storage.

By predetecting a small subset of ions stored temporarily in the multipole guide, the system receives even before a mass spectrometric analysis the information, whether or not the quantity of ions will be sufficient to fill the ion trap optimally. The calibration of the system is performed using the correlation between the ions transferred into the ion trap with the ion signal from the predetector. The optimum number of ions in the trap can be determined considering the signal intensity and the extent of the frequency shift in the FTICR trap.

Another method based on the present invention is that the second multipole ion guide (10) in the system described in Fig. 1 is used as the predetector. For this, a subset of the prestored ions are extracted out of the first multipole

ion guide and transferred in the second one. After the detection of the amount of ions the multipole is switched back into its original operation mode as an ion guide.

Fig. 2 shows the same LDI source with a multipole ion
5 guide, again connected to a Fourier transform ion cyclotron resonance mass spectrometer: Also in this setup, the laser beam (3) goes through an adjustable attenuator (5) a lens (6) for focussing and through the laser window (7) onto the sample (1) in the vacuum system of the mass spectrometer.
10 Also here, the ions are generated from the sample (1) and temporarily stored in the ion guide (8). In this example the ion guide consists of an octopole. After a storage time in this multipole, by reversing the polarity of the voltage at the apertured lens (9), they are transferred through the
15 ion optical lenses (15 and 16) into the ion transfer system (17) of the FTICR mass spectrometers. The ion transfer system in this example consists of several cylindrical ion-optical components, with the aid of which the ions are transferred into the ICR trap (18) in order to be detected
20 mass spectrometrically. The ICR trap is located in mass spectrometric vacuum system within a superconducting magnet (19). All pumping connections of the differentially pumped vacuum system (20) in the Figure have the number 14.

The pre-measurement for determining the number of the ions
25 temporarily stored takes place as follows: A subset of temporarily stored ions is pulsed out of the multipole ion guide, and at the same time, a differential voltage is applied between the two halves of the lens (15). In this way, the ions are deflected to the side, whereupon they hit
30 the wall of the cylindrical ion lens (16) which now operates as ion detector. The current measured here indicates the filling level (of the multipole ion guide)

and used to control the length of the extraction pulse at the extraction electrode (9).

The temporary storage of ions in the multipole ion trap naturally permits also to transfer a larger number of ions at once into the ICR trap, if this is necessary. For instance, in order to isolate only a certain type of ions in the ICR trap, an initial overfilling of the trap is necessary. Consequently, when all other ions are ejected (ion isolation experiment) this ion type will have optimum number of ions in the ICR trap. By knowing the ionic distribution of the sample from a previous mass spectrum, the degree of the overfilling required for the process can be determined.

The temporary storage of the ions in the multipole ion guide allows during the storage time the transfer of the excess kinetic energy by collisions to the gas molecules in the environment and therefore leads to a cooling of the ions. The low energy ions can be transferred into the ICR trap and captured there much more successfully.

Depending on the pressure conditions prevailing in the LDI source, the end plate of the multipole ion guide can be built in form of a skimmer. This allows to create a differentially pumped system and have an higher pressure in the ion source than the rest of the mass spectrometric vacuum system.

A further method based on the present invention is, that for the pre-measurement of the ions, part of the ions stored in the multipole ion guide are pulsed past through the ICR trap, without being captured. These ions hit an ion detector behind the ICR trap and generate a reference signal for the filling level.

In the FTICR mass spectrometry a pre-programmed pulse sequence is used, whereby a so called quench pulse is applied in order to "clean" the trap before every ion generation pulse. A slightly higher voltage (-20 to -50V) is applied on one of the trapping plates for a short time (usually 50 milliseconds), as a result of which the remaining positive ions fly to this plate and get neutralised. Negative ions fly with the same quench pulse to the other electrodes of the ICR trap and thus get also neutralised and eliminated. Based on this quench pulse, a further method can be introduced for the pre-measurement: A subset of the ions temporarily stored in the multipole ion trap can be transferred into the ICR trap and using a quench pulse accelerated to one of the trapping plates, where they hit and generate an (electric) current, which serves as a filling level signal.

Fig 3 shows another setup for a pre-measurement of the amount of the ions stored in the multipole ion guide in an FTICR mass spectrometer with an ion detector behind the ICR trap.

In case of MALDI, in addition to the analyte ions, also excessive amounts of matrix ions are formed. These ions are also stored in the multipole ion guide and afterwards transferred into the ion trap using the ion transfer system. Since additional electric charges solely contribute to the space charge, it is advantageous to remove these, before they even sent into the trap. For this process the multipole ion guide can also be used.

The widespread method of quadrupole mass spectrometry is based on the fact that ions in a "quadrupole filter" can be eliminated or "filtered out" by instable trajectories. The book „Quadrupole Mass Spectrometry" by Peter H. Dawson

(Elsevier 1976) describes on pages 19-35 the operation of a quadrupole as mass filters. Although the filter properties of the higher multipoles (hexapole, octopole) not as good as those of a quadrupole, the ions can be nevertheless
5 filtered in these multipole ion guide. Particularly, elimination of small ions (below a predefined mass to charge ratio) can easily be achieved by selecting the applied high frequency amplitude.

The ion trapping properties of the multipoles improve with
10 increasing number of poles. Therefore, the hexapole and octopole ion guides are as linear ion traps more suitable than the quadrupoles.

Fig. 4 shows a setup containing an electrospray source with spray needle (nebuliser jet) (23) with the electrospray
15 capillary (24) made of glass with metallised ends (25 and 26) and the skimmer (27). This source is connected (29) to a liquid chromatograph (28) generates ions for a Fourier transform ion cyclotron resonance mass spectrometer. The connection (30) of the nebuliser gas is in the carrier
20 platform of the nebuliser jet (20). The vacuum system (31) is pumped differentially here. Each vacuum stage has separate pumping connections (14). The temporary storage and pre-measurement is performed analogous to the case with the LDI source from the storing multipole ion guide.

25 Since the electrospray source is driven continuously, the multipole ion guide is constantly refilled, while using a subset of extracted ions the pre-measurement is performed. However, the pre-measurement only takes a very short period of time, which remains in the microsecond region, so that
30 the inaccuracy in calculating the number of ions based on the pre-measurement is negligible.

Fig. 5 shows an algorithm for the regulation procedure for filling the ICR trap with the desired number of ions. Ions are desorbed for example using a laser pulse and trapped in the multipole ion guide. A pre-measurement establishes if the number of ions in the multipole ion guide is in the range of tolerance. The number of ions has to be large enough to generate a mass spectrometric signal with a good signal-to-noise ratio, but not too large, that the undesirable space charge effects appear in the ion trap. If the number of ions is in the right range, the ion cloud in the temporary storage is transferred into the ion trap for mass spectrometric analysis. If the number of ions is too low, the laser is re-activated and the desorbed ions added to the ones already in the temporary storage. The pre-measurement may now indicate a number of ions, which is in the tolerance range. If not, the procedure is repeated. Ultimately the ions are transferred into the ion trap and analysed mass spectrometrically. If, however, the pre-measurement indicates that the number of the temporarily stored ions is too high, only a certain amount of these ions can now be sent to the ion trap and analysed there. For this purpose, the length of the ion extraction pulse is reduced according to a predefined algorithm. The calculated extraction pulse duration ensures that the number of ions transferred into the ion trap remains in the tolerable range. The connection between the extraction pulse length and the filling level can be determined experimentally.

It is also possible, what fraction of the temporarily stored ions of a "filling" is transferred into the ion trap. In this way, for example, quantitative statements can be made about the ions desorbed with each laser pulse,

although probably not the complete amount of the desorbed ions has been analysed.

A use of the multipole ion guide directly after the LDI or MALDI ionisation as a pre-trap for ion storage, ion
5 filtering and pre-measurement of the total ion charge will allow a complete control over the space charge effects in the trap. In the present invention, one of the most important points in measuring for controlling the number of ions is that not the complete amount of the temporarily
10 stored ions is used. After a pre-measurement for determination of the number of temporarily stored ions, in case of the presence of a large quantity of ions, the remaining ions can be used for a larger number of mass spectrometric analyses in the ICR trap.

Claims

1. A method for controlling the filling of the ion trap of an ion cyclotron resonance mass spectrometer used for mass spectroscopic analysis, which method
5 comprises storing the ions temporarily in a multipole ion guide, determining the filling level of the ion guide by measuring a subset of the temporarily stored ions, and thereafter controlling the filling of the ion cyclotron resonance trap from the ion guide for
10 the mass spectrometric analysis in dependence on the filling level of the ion guide.
2. A method as claimed in Claim 1, wherein the ions which are stored temporarily in the multipole ion guide are mass selectively filtered there by operating the
15 multipole ion guide as a multipole mass filter.
3. A method as claimed in Claim 1, wherein the ions are produced using a laser desorption ion source and wherein the ions are desorbed by the laser pulse on a sample directly into the multipole ion guide.
- 20 4. A method as claimed in Claim 3, wherein light ions derived from the matrix employed in the laser desorption process are filtered out to leave heavy analyte ions in the ion guide.
5. A method as claimed in Claim 1, wherein the ions are
25 formed by electrospray ionisation, and are transferred through systems for removing the solvent and reducing the pressure to a multipole ion guide and stored there temporarily.
6. A method as claimed in Claim 1, wherein the subset of
30 ions for determining the number of ions stored temporarily in the multipole ion guide is transferred

to a detector which is positioned behind the multipole ion guide.

7. A method as claimed in Claim 6, wherein the detector is a secondary electron multiplier.
- 5 8. A method as claimed in Claim 1, wherein the subset of ions for determining the number of ions stored temporarily in the multipole ion guide is transferred into a second multipole, which is operated as a measuring electrode.
- 10 9. A method as claimed in Claim 1, wherein the subset of ions for determining the number of ions stored temporarily in the multipole ion guide is transferred into the ion cyclotron resonance trap and is determined by mass spectrometric measurement.
- 15 10. A method as claimed in Claim 1, wherein the subset of ions for determining the number of ions stored temporarily in the multipole ion guide is transferred into the ion cyclotron resonance trap, wherein an electrical total ion current measurement is performed
20 using an ion quench pulse at one of the electrodes in the ion cyclotron resonance trap.
11. A method as claimed in Claim 1, wherein the subset of ions for determining the number of ions stored temporarily in the multipole ion guide is transferred
25 through the ion cyclotron resonance trap to an ion detector behind the trap.
12. A method for controlling the filling of an ion trap substantially as herein before described with reference to and as illustrated by the accompanying
30 drawings.

13. An ion cyclotron resonance mass spectrometer for mass spectroscopic analysis using an ion trap filling method as claimed in any one of Claims 1 to 12, having an ion source, an ion trap, and a multipole ion guide disposed between the ion source and the ion trap, wherein the apparatus includes means for controlling the multipole ion guide so as to store ions temporarily therein, means for measuring a subset of the temporarily stored ions to determine the filling level of the ion guide, and means for controlling the filling of the ion cyclotron resonance trap, in dependence with the said determination.
14. A device as claimed in Claim 13, including an ion detector behind the ion cyclotron resonance trap, for measuring ions flying thorough the trap.
15. A device as claimed in Claim 13 or Claim 14, including an end plate in form of an apertured plate for storing the ions, and a switchable potential supply for the apertured plate, whereby the switchable potential is such as to provide either a reflection potential for storing the ions or an extraction potential for removing the ions.
16. A device for the temporary storage of ions formed in a laser desorption processes, which device comprises a sample carrier plate for laser desorption, a multipole ion guide directly in front of the carrier plate, an apertured end plate for storing the ions, and a switchable potential supply for the apertured end plate, whereby the switchable potential provides either a reflection potential for storing the ions or an extraction potential for removing the ions.

17. A device for temporarily storing the ions for the method described in Claim 1, which comprises a sample carrier plate for laser desorption, a multipole ion guide directly in front of the carrier plate, an end plate in form of an apertured plate for storing ions, and a switchable potential supply for the apertured plate, whereby the switchable potential provides either a reflection potential for storing the ions or an extraction potential for removing the ions.
18. A device for measuring the number of ions from a subset of the temporarily stored ions as in Claim 1, 6, and 11, wherein an ion detector is placed behind the ion cyclotron resonance trap, with which the ions flying through the trap can be measured.
19. An ion cyclotron resonance mass spectrometer for carrying out a method according to any one of Claims 1 to 12, substantially as hereinbefore described with reference to and as illustrated by Figure 1, Figure 2, Figure 3, or Figure 4 of the accompanying drawings.
20. A method for controlling the filling of the ion trap in ion cyclotron resonance mass spectrometers, equipped with multipole ion guides, with desired number of ions, wherein the ions are first stored in a multipole ion guide and then the filling level of the ion guide is determined by measurement of a defined subset of these temporarily stored ions before the ion cyclotron resonance trap is filled from the ion guide for the purpose of mass spectrometric analysis depending on the filling level of the ion guide and therefore in a controlled way.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.